



0107-020P

Agent for treating septicemia, process for preparing and therapeutic process

Field of invention

5 The invention relates to a therapeutic agent for the treatment of septicemia, its preparation and use in the pharmaceutical industry and in medicine.

Background

10 Septicemia with its frequently lethal complications is one of the most feared clinical syndromes in medicine. It is therapeutically not controllable, claiming hundreds of thousands of casualties a year in Western countries alone. New treatment options have to be found because antibiotics act too slowly, and do not prevent the release of bacterial toxins and partly even intensify it. Pouring out of messenger substances (cytokines) by the host organism released by bacterial toxins is the most important element of the pathogenetic cascade leading to the clinical evolution of septicemia. Various new therapeutic
15 approaches, as blocking of bacterial lipopolysaccharide (LPS) as the most important toxin by antibodies or antagonizing the endogenous, so-called proinflammatory cytokines failed completely in comprehensive clinical studies (C. Natanson et al., Ann. Intern Med. 120, 771-783 (1994)).

Summary of the invention

20 It is an object of the present invention to develop a therapeutic agent for the treatment of septicemia. In accordance with the present invention, it was discovered that highly dosed LBP suppresses the synthesis of an important septicemia mediator molecule caused by the bacterial toxin LPS, namely TNF. The production of this TNF protein and
25 other mediators is suppressed in the mouse by the addition of LBP. In addition, liver damage induced by the addition of LPS is prevented by LBP and the number of surviving animals significantly increases. That means that the addition of LBP protects against the effects of LPS during septicemia, which represents a new therapeutic process for treating septicemia. Thus, the above object was accomplished by an agent containing the protein
30 LBP binding lipopolysaccharide, its mutants, variants or hybrid proteins, as basic active component. Human and murine such as rat, or rabbit LBP are also effective for the purpose.

Brief description of the drawing

The invention is disclosed with reference being had to the drawing, wherein:

5 Fig. 1 shows stimulation of a murine macrophage cell line *in vitro* with various concentrations of the bacterial toxin LPS for synthesizing the septicemia mediator TNF depending on LBP;

Fig. 2 shows how due to the LBP contained in serum, the addition of LBP in the presence of serum suppresses the production of TNF by the macrophage cell line;

10 Fig. 3 shows the suppression of TNF syntheses as the concentration of serum is increased with the added LBP remaining constant;

Fig. 4 shows that LBP levels of mouse produced by an exogenous addition of LBP correspond to the acute physiological phase levels produced by addition of LPS;

15 Fig. 5(a) shows with TNF that cytokine release induced by LPS can be suppressed in the mouse by the simultaneous addition of LBP;

Fig. 5(b) shows that with IL-6 cytokine release induced by LPS can be suppressed in the mouse by the simultaneous addition of LBP;

Fig. 6 shows that in addition, the liver damage caused by LPS and detected by increasing ALT enzyme levels, are suppressed by simultaneously adding LBP; and

20 Fig. 7 shows that the addition of LBP significantly reduces the lethality in a LPS septicemia model, carried out with 20 mice per group.

Detailed description

25 The structure of LBP is known. It was obtained by isolating a clone from an acute phase cDNA gene bank and subsequently sequencing and deriving the amino acid sequence. Recombinant LBP is prepared by cloning cDNA in an expression vector and co-infection of insect cells with the baculovirus.

30 The cloned protein (LBP) binds LPS with high affinity and is secreted into serum as acute phase protein during septicemia. As is shown in the figures, LBP inhibits the effects of LPS and can –if given to mice – suppress septicemia caused by LPS and very significantly reduce lethality. This applies when using LBP simultaneously with the onset of septicemia and also before. Thus LBP appears to be suited for preventing septicemia also in high-risk patients. As LPS plays a central part also in septicemia caused by gram-positive

bacteria and for the systemic inflammatory response syndrome, a clinical picture identical to septicemia caused by trauma, by translocation of gram-negative intestinal flora. The inhibition of LPS effects by LBP can also improve these dramatic clinical syndromes.

5 Apart from the highly active recombinant LBP optimized mutants the function of which was modified and which are also used as therapeutic agents for the treatment of septicemia, are equally suited for implementing the present invention.

10 A further basic possibility for implementing the present invention is to clone the LBP gene in an adenoviral vector with high activity in the liver behind the strong CMV promoter to achieve high levels of expression in addition to the intrinsic hepatic expression of LBP.

15 The invention can be suitably applied in the case of septicemia caused by gram-negative and gram-positive bacteria; and in the case of systemic inflammatory response syndrome (SIRS) caused by trauma and injury.

20 When no high LPS concentration is present, then it was found that LBP does not affect TNF syntheses. However, the stimulation of macrophages by lower LPS quantities is inhibited by high LBP concentrations as they occur *in vivo* during the acute phase and can also be achieved by an exogenous addition of LBP.

25 In accordance with this example of obtaining LBP, the complete LBP cDNA is cloned in the pACHLT-B vector (Pharmingen, San Diego, USA) behind the strong polyhedrin promoter and behind glutathione S-transferase (GST) cDNA to express a GST fusion protein. Then a 500 ml cell culture of Sf-9- insect cells is infected with this vector and the baculovirus DNA (Baculogold, Baculovirus DNA in a linearized form, also from Pharmingen, San Diego, USA). After 2 days the cells are subjected to lysis and the lysate is coupled to glutathione sepharose in the presence of triton X-100 in a "batch" process.
30 Then LBP is split off by the participant in the fusion by digestion with thrombin followed by a treatment with calbisorb to remove triton and a treatment with benzamidine sepharose to remove the thrombine residue. The resulting concentrations of pure LBP totals 0.3 – 0.5 mg/ml.